Suppression of Floral Induction by Inhibitors of Steroid Biosynthesis [1, 2]

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This paper concerns a class of metabolic inhibitors which possess the ability to suppress the flowering of short-day plants and which appear to do so by interfering with generation of the flowering stimulus by the leaf. The inhibition of flowering with which we are here concerned is thus different from that elicited by 5-fluorouracil and 5-fluorodeoxyuridine. These two substances act by preventing successful receipt by the bud of the leaf-produced floral stimulus (4, 17). The substances here reported as active in inhibition of the generation of flowering stimulus by the leaf are of further interest in that they are inhibitors of the biogenesis of steroids.

Materials & Methods

Plant Material. The cocklebur (*Xanthium pensylvanicum* Wall) plants used were of our inbred strain. They were grown and handled as earlier described (4). The temperature during an inductive 16-hour night was kept at 23 to 25 C. After 9 days the apical buds were dissected and classified according to Salisbury's floral system (14). With this method the standard deviation of the stage based on a group of 15 untreated plants is of the order of 15 % of a floral stage unit (table I).

Seedlings of *Pharbitis nil* Chois, strain Violet, were germinated for four or five days under artificial light and controlled conditions as earlier described (17). For floral induction the plants were exposed to one 16-hour dark period at 28 to 29 C. After ca. two weeks the number of flower buds per plant developed in response to such an inductive night was determined (17).

Steroid Inhibitors. The following substances, all known to be inhibitors of cholesterol biosynthesis (10), and all obtained from Smith, Kline, & French Laboratories, Philadelphia, were tested for their effects on photoperiodic induction: SK&F 525-A (β-diethylaminoethyldiphenylpropyl acetate hydrochloride); SK&F 3301-A (2,2-diphenyl-1-(β-dimethylaminoethoxy)-pentane hydrochloride); SK&F 7732-A_3 (tris-(2-dimethylaminoethyl)-phosphate trihydrochloride), and SK&F 7997-A_3 (tris-(2-diethylaminoethyl)-phosphate trihydrochloride).

Triparanol (MER/29) citrate, 1-[(4-diethylaminoethoxy)-phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl)-ethanol was obtained from the Wm. S. Merrell Co., Cincinnati.

The inhibitors were dissolved in distilled water. The pH of the solution was immediately adjusted to 7.2 in the case of SK&F 7732 and SK&F 7997, or to pH 6.5 for the other materials. One drop of Tween 20 was added per 100 ml solution. Freshly prepared solutions were used for each experiment.

The inhibitors were applied by dipping leaves or tips of cocklebur or cotyledons of Pharbitis into the appropriate solution. Plumules of Pharbitis seedlings were treated by application of 0.01 ml of the inhibitor solution with the aid of a microsyringe.

Water-insoluble steroids were dissolved in absolute ethanol. To this solution, water containing Tween 20 was added with vigorous stirring to yield a final ethanol concentration of 80 %. Xanthium leaves dipped in such an emulsion of 80 % ethanol were not damaged.

Labeled Precursors. Sodium acetate-2-C^{14} (California Corp. for Biochemical Research, Spec. act. 20.5 mc/mmole) was dissolved in distilled water to the required concentration.

Mevalonic acid-2-C^{14} (N,N'-dibenzyl ethylenediamine salt) (Tracer Lab. Inc., Spec. act. 3.16 mc/mmole for salt, 1.58 mc/mmole for free acid) was prepared in aqueous solution as described by Purcell et al. (12).

Treatment of Plants With Labeled Metabolites. Three groups of 25 Xanthium plants each were used for each experiment. These will be referred to as "induced", "vegetative," and "inhibited." The plants were defoliated to the one most sensitive leaf (approximately one-half expanded). The apical bud functioned as receptor. The leaves were first dipped in an aqueous solution of Tween 20 and then allowed to dry. The leaves of the inhibited group were next dipped into a 2 mg/ml solution of SK&F 7997 or other inhibitor. On all plants a ring of vaseline was applied around the petiole near the leaf blade to prevent the labeled metabolite from spreading along the petiole to the bud. As soon as the leaves treated with inhibitor had dried, application of the labeled compound was started. To the labeled material dissolved in water, Tween 20 and 2 % ethanol were added as

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detergents. Three times 0.1 ml of the radioactive solution was applied with a syringe per leaf blade—a total of 1 μc of mevalonic acid (MVA)-2-C^{14} or 6.6 μc of acetate-2-C^{14}—per leaf. Finally, an application of water containing Tween 20 and 2 % ethanol was made. Drops put on leaf blades previously wetted with an aqueous Tween 20 solution spread out evenly over the blade, so that the whole leaf surface is covered with a thin film of liquid after the application of 0.1 ml per leaf. This method results in an effective penetration of the radioactive material into the leaf. When the leaves were rinsed in distilled water at the time of harvest, always less than 3 % of the applied radioactivity was removed by such rinsing. In addition, although determination of the radioactivity of a labeled leaf with a Geiger counter on the upper and lower side of a leaf blade immediately after application of the label indicated much more radioactivity on the upper side than on the lower, this difference disappeared within 16 hours.

Buds were labeled by the application of 0.01 ml per bud of the metabolite solution.

Two to three hours after the labeled material had been applied, the plants were exposed to a 16-hour dark period. The vegetative group received a 10-minute night interruption from incandescent lamps exactly 8 hours after the beginning of darkness. This treatment completely suppressed flowering.

At the end of the long night or several hours later as indicated below the leaf blades and buds were harvested separately, frozen in liquid nitrogen and lyophilized. The dry material was stored in a freezer at −20 C until further processing.

In all labeling experiments controls of each group were kept to check the effectiveness of the long night treatment in inducing flowering, and of the night interruption and SK&F 7997 treatments in suppressing flowering. In all experiments each control exhibited the appropriate and expected response.

Extraction Procedure. The dry plant material was ground and hydrolyzed with boiling 3 N HCl. The hydrolysate was filtered and the residue continuously extracted with methanol. Acid-soluble material was removed and the remainder (neutral & acidic lipids) fractionated by alumina chromatography (2).

Specific example: The lyophilized leaves (buds) of 25 plants, dry weight 3.4 g (0.2 g), were refluxed with 150 ml (10 ml) 3 N HCl for 2 hours. The hydrolysate, after cooling, was filtered and the residue washed free of acid (filtrate pH > 6). The air-dry residue together with the filter paper, and the cotton pledget used for mopping the Buchner funnel, were placed in the extraction thimble of a Soxhlet extractor. Following continuous extraction with 100 ml (20 ml) of absolute methanol for 18 hours (4 hr) the methanol extract was transferred to a separatory funnel with one volume of dichloromethane. One volume of 3 N HCl was added and the lower phase withdrawn into a second separatory funnel containing one volume of water. The extraction was repeated with one-volume portions of CH_2Cl_2, each extract being passed through the same wash water. The combined CH_2Cl_2 extracts were dried over Na_2SO_4 and the solvent evaporated, leaving 353.2 mg (24.2 mg) of residue. This was applied to a column of 15 g (1 g) neutral alumina (Woelm, Eschwege) grade III. The following 50 ml (3 ml) portions of eluate were collected: Fractions 1–2, 5 %; 3–4, 10 %; 5–6, 25 %; 7–8, 50 % benzene in hexane; 9–10, benzene; 11–12, 5 %; 13–14, 10 %; 15–16, 25 %; 17, 50 % benzene in ether; 18, ether and 1 %; 19, 2.5 %; 20, 5 %; 21, 10 %, and 22, 20 % methanol in ether.

An aliquot of each fraction, weight less than 1 mg (or the entire fraction) was plated on an aluminum planchet and radioactivity determined under a micromil window tube in an atmosphere of Q gas on an automatic sample changer. Total or specific radioactivities were plotted against fraction numbers as shown below.

Results

In preliminary experiments, selected inhibitors of cholesterol biosynthesis were applied to half-expanded Xanthium leaves at the beginning of a 16-hour inductive dark period. Application of either SK&F 525 or SK&F 3301 resulted in yellowing of the treated leaves. The symptoms became visible two to three days after treatment and increased in intensity up to ten days after treatment. With an inhibitor concentration of 2 mg/ml more than half of the leaf blade became yellow within ten days. The yellowing was restricted to the treated leaves. Untreated leaves on the same plant showed no symptoms. In addition, if only half of a blade (one side of the midrib) was treated, the other half remained green. If the apical half of a leaf was treated, the basal half remained green. If the basal half of a leaf was treated, some yellowing was usually observed along the veins of the untreated apical half, suggesting that some of the inhibitor had moved in the transpiration stream. Fully mature leaves showed even more severe symptoms as a result of treatment with SK&F 525 and SK&F 3301 than half-expanded leaves.

SK&F 525 only inhibits flowering slightly if applied in concentrations up to 2 mg/ml. SK&F 3301 inhibits flowering much more strongly. Thus a concentration of 2 mg/ml of the latter substance caused 85 % inhibition of flowering when applied before the inductive dark period.

Triparanol applications had no significant effect on floral induction in Xanthium if the substance was applied one day before or shortly before the long night and in concentrations up to 2.5 mg/ml (table I). Necrotic spots appeared on the leaf blades four to five days after treatment with triparanol, so that ten days after treatment more than 50 % of the leaf blade had become necrotic. These symptoms were again restricted to the treated leaf.

More interesting results were obtained with the

inhibitors SK&F 7732 and SK&F 7997. Both compounds are inhibitory to floral induction if applied in a concentration of 2 mg/ml before the beginning of a 16-hour dark period. These compounds cause no noteworthy damage to the leaves. After application of very high concentrations a few small necrotic spots may develop in the treated leaf blade and the expansion of the blades is slightly inhibited. As compared to the effects of SK&F 525, SK&F 3301, and triparanol the vegetative symptoms caused by SK&F 7732 and 7997 are mild, although these materials completely suppress floral induction. The further work reported below is restricted to the compound SK&F 7997.

Kinetics of Inhibition of Floral Induction by SK&F 7997. Figure 1 shows the effect of different concentrations of SK&F 7997 on the flowering response of Xanthium. In concentrations up to 1 mg/ml its inhibitory effect is slight, but at higher concentrations the inhibition is significant. In many experiments a concentration of 2 mg/ml, applied 2 hours before the long night, completely suppressed flowering. Results of a representative experiment in which SK&F 7997 was applied either to Xanthium leaves or to apical buds at different times before, during and after a long night are presented in figure 2. It is clear that application of the material to the apical bud is without effect on flowering. SK&F 7997 therefore inhibits the flower-inducing processes only if applied to the leaf. Application shortly before the beginning of the dark period is the most effective. The extent of inhibition gradually decreases as applications are made later during the dark period and applications made after the end of a 16-hour dark period are without flower-inhibiting effect.

If SK&F 7997 is applied 26 hours before the inductive long night, its inhibitory effects on flowering are negligible. (table I). This indicates that the inhibitor is active for only a brief period. It is possibly metabolized or stored in such a way as to render it inactive.

Results similar to those presented above for Xanthium have also been obtained with Pharbitis. With this plant SK&F 7997, applied to the cotyledons in a concentration of 1 mg/ml, fully suppresses flowering. As is shown in figure 3, in Pharbitis also application of the inhibitor to the cotyledons before the

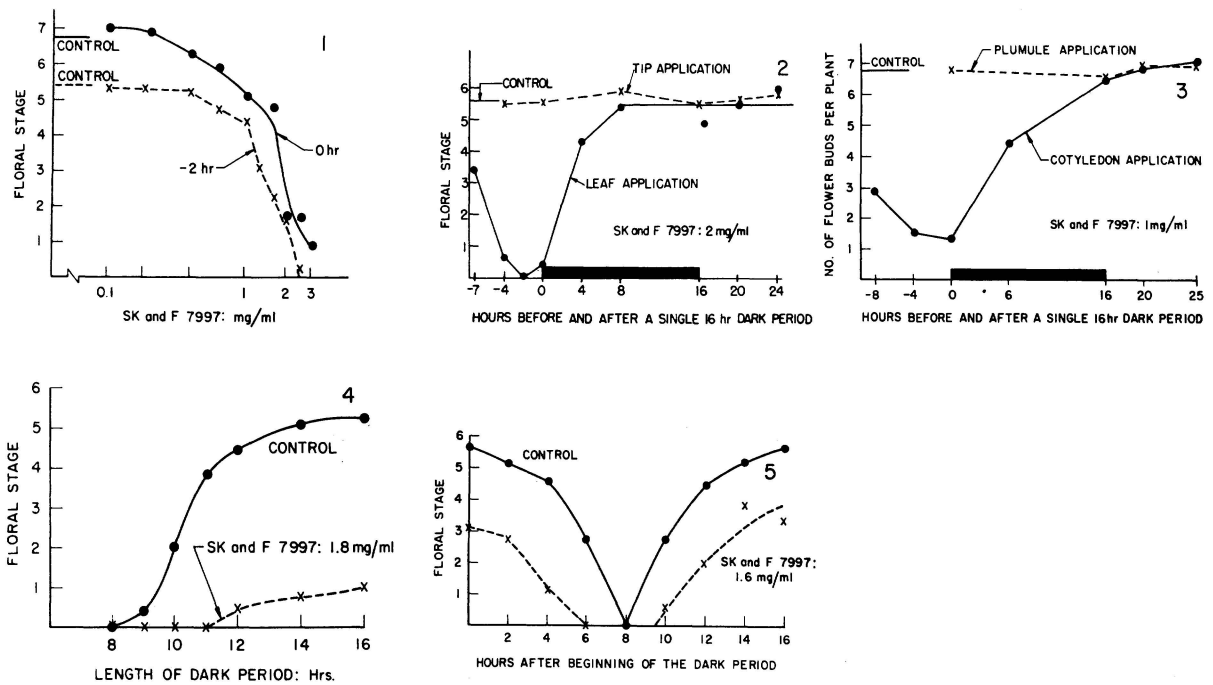

Fig. 1. Effects of various concentrations of SK&F 7997 on the flowering response of Xanthium. Data for two different experiments in which the inhibitor was applied to leaves two hours or immediately before the 16-hour dark period. Fifteen plants per treatment.

Fig. 2. Effect of applying SK&F 7997 to Xanthium leaves or buds at different times before, during and after an inductive dark period on floral response. Fifteen plants per treatment.

Fig. 3. Effect of applying 1 mg/ml SK&F 7997 to cotyledons or plumules of Pharbitis at different times on flower formation. Seventeen plants per treatment.

Fig. 4. Effect of SK&F 7997 on the critical dark period in Xanthium. Inhibitor was applied before darkness. Fifteen plants per treatment.

Fig. 5. Effects of a 1-minute night interruption, applied at different times during a 16-hour inductive dark period, on the flowering response of Xanthium, treated with 1.6 mg/ml SK&F 7997. Leaves treated with inhibitor 2 hours before the dark period. Light for the night interruption was supplied by five 200-w incandescent bulbs at a distance of 75 cm from the leaves. Ten plants per treatment.

Table I

Effects of SK&F 7997 & Triparanol on Floral Induction of Xanthium by a Single 16-Hour Dark Period

Inhibitor	Interval between application & dark period		Control (No inhibitor)
	26 hr	2 hr	
SK&F 7997	5.0 ± 0.00	1.1 ± 0.36	5.3 ± 0.13
SK&F 7997	4.5 ± 0.21	0.8 ± 0.37	4.9 ± 0.07
Triparanol	5.7 ± 0.13	5.2 ± 0.22	5.4 ± 0.16

Floral stages as determined by dissection 9 days after end of the long night. Both inhibitors were applied in a concentration of 2 mg/ml and to the leaves only. Fifteen plants per treatment.

long night is most effective in suppression of flowering.

It is of interest to determine which of the processes of photoperiodic induction is inhibited by SK&F 7997. In Xanthium the critical dark period is somewhat lengthened in the presence of the inhibitor (see fig 4). This is not, however, to be considered as evidence that the inhibitor affects the timing mechanism, since with dark periods close to the critical, the flowering response is small, and, in general, small responses are more easily suppressed than large ones. A more rigorous experiment was, therefore, carried out, following Salisbury's technique (15). Individual groups of treated and untreated plants were exposed to 1 minute of light at 2-hour intervals during a 16-hour night. As is shown in figure 5, there is no indication that the portion of the dark period most sensitive to light interruption is displaced in the presence of SK&F 7997, as it is in the presence of Co^{++} (15). SK&F 7997 does not, therefore, affect the timing mechanism. Since the inhibitor functions in the leaves and during the dark period, the most obvious conclusion is that SK&F 7997 may inhibit flower hormone synthesis. Since the substance is an inhibitor of steroid biogenesis in animal tissues (10), and since, as will be shown below, it also inhibits steroid biosynthesis in leaves, the hypothesis suggests itself that the flower hormone may be a steroid. A wide variety of steroids and steroid precursors have, therefore, been tested for ability to overcome the inhibition of flowering caused by SK&F 7997. In all of these experiments, the inhibitor was applied 2 hours before the beginning of the dark period and the test substances were repeatedly applied as an emulsion during the dark period. Among the compounds tested in this way were: mevalonic acid, farnesol, squalene, β-sitosterol, stigmasterol, lanosterol, desmosterol, tomatine, holothurin, estrone, estrone sulfate, dehydroepiandrosterone, and diosgenin. None of these substances proved capable of overcoming the inhibition of flowering exerted by SK&F 7997. Nonsaponifiable lipids of Xanthium were also tested for activity in reversal of SK&F 7997 inhibition. For this purpose large quantities of individual chromatographic fractions (see below) of neutral and acidic nonsaponifiable lipids were prepared by working up 225 g of lyophilized induced Xanthium leaves. None of the fractions proved capable of reversing the inhibition caused by SK&F 7997 or to induce floral initiation in vegetative Xanthium plants. It has not, therefore, been possible to counteract the inhibition of induction caused by SK&F 7997.

The problem of the mechanism by which SK&F 7997 inhibits floral induction has been further studied by the use of labeled precursors of steroids, namely acetate and MVA. The general procedures followed have been outlined under Methods.

In a typical labeling experiment, 6.6 μc of acetate-2-C^{14} were administered per leaf. Leaves and buds were harvested separately, 26 hours after the beginning (or 10 hr after the completion) of the long night. The elution pattern for the lipid extract of the leaves is shown in figure 6. There is no apparent difference between patterns produced by induced and vegetative leaves with the exception that in the former the radioactivity of Fractions 1 and 2 is greater (see legend to fig 6). The radioactivity present in fractions from inhibited leaves is generally less than that present in the corresponding fractions of the other two groups (especially in Fract. 11). The buds also contain radioactive lipids and the elution patterns are shown in figure 7. Again, the activity present in inhibited plants is less than that in the other two groups, which do not however exhibit any consistent differences.

In a similar type of experiment, 1 μc MVA-2-C^{14} was applied per leaf and the leaves harvested immediately at the end of the 16-hour dark period. Figure 8, where specific radioactivity is plotted against fraction numbers, shows several peaks (Fractions 8 & 9 are probably alike). The most striking difference between the elution patterns of the three groups is the very high specific activity of Fractions 14 and 16 in inhibited plants. The radioactivity present in the buds was too small to yield meaningful elution patterns.

When Fraction 14 (4×10^4 cpm), isolated from 75 plants treated with SK&F 7997 and labeled MVA, was applied to 10 Xanthium leaves, it was metabolized. The elution pattern of the products is shown in figure 9. There is no difference between the elution pattern from these leaves and that yielded by a group of ten similarly treated plants, which had been kept in the vegetative state by light interruption of the dark period. Fraction 16 was metabolized to a lesser extent. The buds also contained a number of labeled metabolites after application of Fraction 14 to the leaves.

Three groups of Pharbitis seedlings, each containing 100 plants, received 0.25 μc MVA-2-C^{14} via the cotyledons. The plumules together with small portions of petioles and hypocotyls were harvested after 20 hours of darkness. By this time the flower

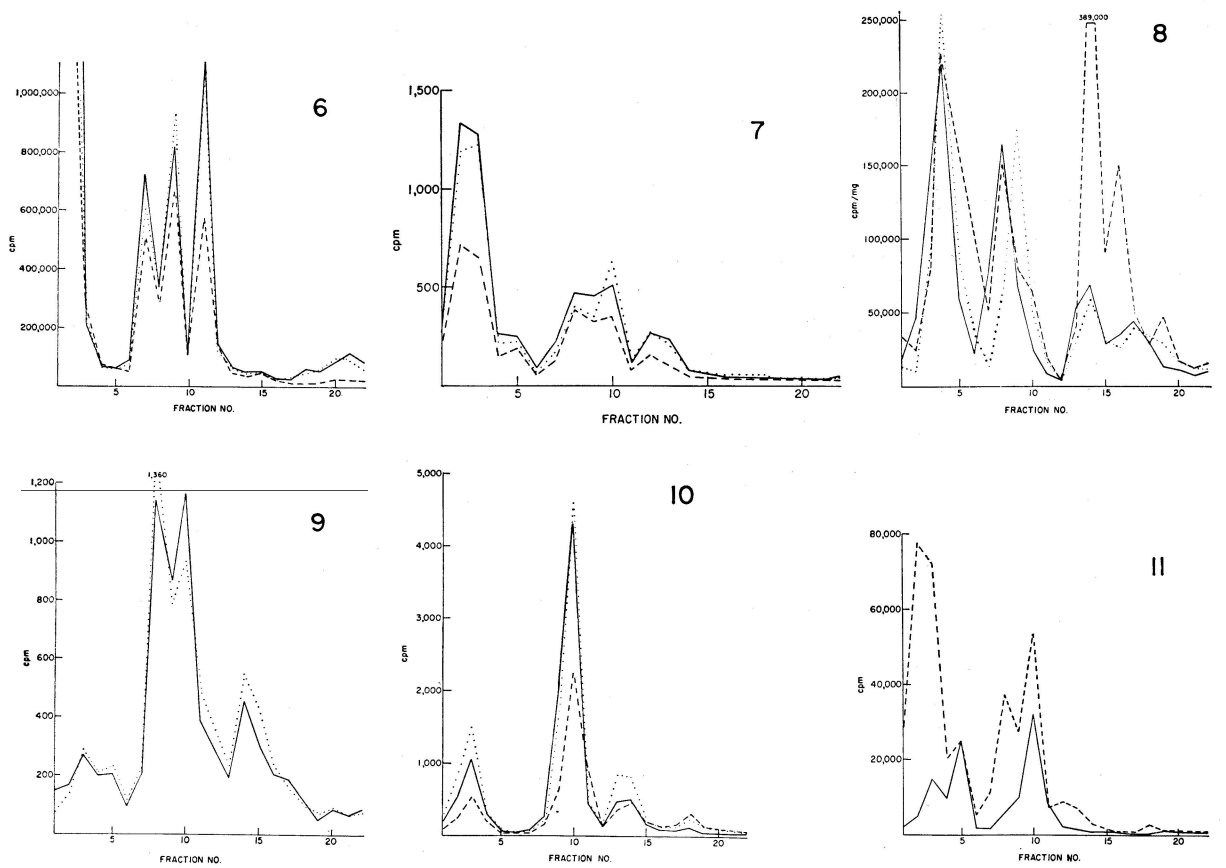

Fig. 6. Distribution of radioactivity among chromatographic fractions of Xanthium leaf lipids following application of 6.6 μc acetate-2-C^{14} per leaf. Twenty-five plants per group were induced (———), vegetative (. . . .), or inhibited (- - - -). Leaves were harvested 26 hours after beginning of long night (28 hours after application of acetate). The radioactivity in Fraction 1 was 3.23, 1.36, and 2.06 $\times$ 10^6 cpm and that in Fraction 2 was 5.12, 3.50, and 3.35 $\times$ 10^6 cpm for the induced, vegetative, and inhibited groups, respectively.

Fig. 7. Distribution of radioactivity among chromatographic fractions of Xanthium bud lipids following application of 6.6 μc acetate-2-C^{14} to the leaf of each plant (see fig 6).

Fig. 8. Specific activities of chromatographic fractions of Xanthium leaf lipids following application of 1 μc mevalonic acid-2-C^{14} per leaf at the beginning of 16-hour dark period. Twenty-five plants per group were induced (———), vegetative (. . . .), or inhibited (- - - -). Leaves were harvested at the end of the long night.

Fig. 9. Distribution of radioactivity among chromatographic fractions of Xanthium leaf lipids following application of Fraction 14 (4 $\times$ 10^4 cpm), isolated from 75 plants treated with SK&F 7997 and MVA-2-C^{14}. Ten plants per group were induced (————) or vegetative (. . . .). Leaves were harvested at the end of the 16-hour night.

Fig. 10. Distribution of radioactivity among chromatographic fractions of Pharbitis plumule lipids following application of 0.25 μc mevalonic acid-2-C^{14} to the cotyledons of each seedling. Hundred plants per group were induced (————), vegetative (. . . .), or inhibited (- - - -). Plumules were harvested 20 hours after the end of the dark period.

Fig. 11. Distribution of radioactivity among chromatographic fractions of Xanthium bud lipids following application of 0.03 μc mevalonic acid-2-C^{14} (————) or 0.33 μc acetate-2-C^{14} (- - - -) to the bud of each plant. Twenty-five buds per group were harvested 26 hours after the beginning of the long night.

hormone had arrived in the apex of Pharbitis in quantities sufficient to elicit a maximal flowering response (17, 18). The plumules exhibit high radioactivity (fig 10), but again the elution patterns, which are slightly different from those of Xanthium buds, show no significant differences between the induced and vegetative groups. However, the inhibited group contains generally less activity than the corresponding fractions of the other two groups (especially in Fract. 10).

Xanthium buds (biggest leaf smaller than 1 cm) can also incorporate labeled acetate and MVA into nonsaponifiable material. This is shown by an experiment in which 0.03 μc MVA-2-C^{14} or 0.33 μc acetate-2-C^{14} was applied directly to buds of two different groups of 25 Xanthium plants immediately prior to the dark period. The buds were harvested 26 hours later. The elution patterns of figure 11 are similar to those obtained when the label is applied to the leaves.

None of the chromatographic fractions used in these experiments represents a single compound and the nature of the radioactive metabolites has not been elucidated. In a model experiment squalene was found to be eluted in Fraction 1, lanosterol in Fraction 6, farnesol in Fraction 8, and various sterols in Fractions 9 and 10. Since the production of radioactive materials in Fractions 9 and 10 is most suppressed by SK&F 7997, this material has been studied further. Samples of Fraction 9, obtained by alumina chromatography of the extracts from acetate-fed leaves (fig 6) were examined by gas and thin-layer chromatography. The induced, vegetative, and inhibited leaves all contained the same two components. In the gas chromatogram (Johnson, D. F. & E. Heftmann. 1962. Unpublished work), their retention times correspond to β-sitosterol and stigmasterol, respectively. The trifluoroacetates of the two substances exhibited the same mobilities in thin-layer chromatograms (1) and the same sulfuric acid color reactions as the trifluoroacetates of β-sitosterol and stigmasterol, respectively.

Labeled Fraction 9 from vegetative leaves was then combined with the corresponding fraction prepared from a large number of unlabeled Xanthium leaves and crystallized from methanol. The crystalline material was acetylated and one part chromatographed on 1,000 parts of silica (Anasil S, Analabs, Inc., Hamden, Conn.), which had been prewashed with benzene and then hexane. Gradient elution with benzene in hexane yielded fractions of β-sitosterol acetate, followed by fractions of stigmasterol acetate. These were examined by infrared spectroscopy and the spectra found to correspond, band for band, with those of authentic β-sitosterol acetate, and stigmasterol acetate, respectively. The individual acetates were highly radioactive.

Discussion

The experiments reported in this paper supply data on the effects of hypocholesteremic agents on plants. Present knowledge concerning the sites of action of these compounds on cholesterol synthesis has been summarized by Holmes and DiTullio (10). All of these inhibitors block the biosynthetic pathway between MVA and cholesterol and all do so at more than one site. Both SK&F 525 and SK&F 3301 exert their effects at the polyprenol pyrophosphate level and thus prior to formation of the steroid nucleus. Application of either of these compounds to plants causes yellowing of the treated leaves, possibly by blocking of phytol biosynthesis and thus interference with chlorophyll production.

Triparanol, which blocks conversion of desmosterol to cholesterol, causes severe necrosis of leaves, but is without effect on floral induction. Although the action of this compound has not been studied in detail, further studies with it might well yield clues as to roles of sterols in plants.

SK&F 7732 and SK&F 7997 which in vitro inhibit the conversion of lanosterol to cholesterol, scarcely elicit any symptoms in plants other than suppression of floral induction. It is of interest that SK&F 3301 which also blocks the conversion of lanosterol, is likewise a potent inhibitor of floral induction.

The incorporation by Xanthium leaves of labeled acetate and MVA into various nonsaponifiable materials is inhibited by SK&F 7997. The two principal compounds whose production is thus inhibited by SK&F 7997 have been identified as β-sitosterol and stigmasterol, two sterols widely found in higher plants. The evidence is, therefore, good that SK&F 7997 blocks sterol biosynthesis in leaves. The site of the block remains to be elucidated. The precursor which accumulates in Fraction 14 during treatment with SK&F 7997 is readily metabolized, in the absence of the inhibitor, to Fractions 9 and 10 (β-sitosterol and stigmasterol). The precursor is not identical with lanosterol, squalene, or farnesol.

A considerable amount of labeled nonsaponifiable material was detected in the buds of plants whose leaves had been supplied with labeled metabolites. Since the flower hormone is known to move from leaf to bud, it might be hoped that interesting differences between induced and vegetative plants would be easier to detect in buds than in leaves. The direct application of labeled substrate to buds results, however, in considerable conversion to nonsaponifiable material. It seems possible, therefore, that when leaves are supplied with labeled acetate or mevalonate, some precursor, or intermediate, is translocated to the bud and there converted to sterols. To obtain a more direct answer to the question of whether sterols are translocated in the plant, labeled Fractions 9 and 10 (which contain β-sitosterol & stigmasterol) were applied to leaves, and the buds of the treated plants were harvested after one or two days. Such buds always contained labeled material. It seems possible, therefore, that sterols may be translocated in the plant. Another observation which favors this notion is the fact that in plants whose leaves or coty-

ledons were supplied with labeled material, the sterol Fraction 10 of the buds of inhibited plants was always less in amount than in the buds of non-inhibited plants. Since all evidence indicates SK&F 7997 is not transported from the treated leaf and acts only in the treated leaf, the differences observed in the buds must apparently be due to translocation of the final products from the leaves.

SK&F 7997 appears to be a metabolic inhibitor whose use in photoperiodic studies yields some clue as to the nature of the flower hormone. The fact that it inhibits induction in the leaf suggests that the flower hormone may be an isoprenoid- or steroid-like compound. Another possible interpretation is that the inhibitor blocks the production of some co-factor necessary for hormone synthesis. In either case, the effect of SK&F 7997 is clearly associated with flower hormone synthesis.

As has been demonstrated experimentally, biosynthesis of β-sitosterol and stigmasterol is suppressed by SK&F 7997. Both sterols are, however, produced in equal amounts in vegetative and in induced plants. It is not, therefore, through its effects on these sterols that SK&F 7997 inhibits photoperiodic induction. The physiological role of these two sterols, universally present in higher plants (3, 9), remains unknown.

It is possible that the flower hormone is a minor component, contained in some one of our fractions, but present in such small amounts that it has escaped detection. A further possibility is, of course, that SK&F 7997 inhibits photoperiodic induction by blocking some unknown metabolic pathway, different from that leading to sterol production.

Pronounced changes in steroids occur in yucca and agave (11) with approach of the flowering season. This has led Hendricks (8) to put forward the hypothesis that the flower hormone might be a steroid. He also suggested that sterols might be solubilized for transport by glycosidation.

Chouard (5, 6) has shown that applications of estradiol induce flowering in *Callistephus sinensis* grown under short-day conditions, whereas untreated control plants remained vegetative. Estrone was found to be inactive.

Roberts (13) has extracted a lipid material from flowering plants which accelerates floral development, although it does not induce floral initiation under non-inductive conditions.

Sironval (16), working with the long-day plant, *Fragaria vesca,* has prepared an unsaponifiable fraction from leaves of flowering plants. Application of this material to young detached runner plants, not yet fully ripe-to-flower and kept in long days, increased the percentage of flowering plants. The sterol fraction prepared from this crude extract was active only if applied in high concentration. Vitamin E was, however, active in very small amounts. Sironval's test for flower hormone cannot, however, be considered as a reliable one since it consists only of a quantitative increase in flowering in juvenile plants grown under inductive conditions.

Hemberg and Lowén (7) have reported that the number of fruits produced by Datura is increased by application of vitamin K.

Since both vitamins E and K contain isoprenoid side-chains, both were tested in the present experiments. Neither possesses the power to overcome SK&F inhibition or to induce flowering under non-inductive conditions, with either Xanthium or Pharbitis.

Summary

This paper reports the symptoms elicited by a number of inhibitors of cholesterol biosynthesis and the effects of these inhibitors on photoperiodic induction in the short-day plants, *Xanthium pensylvanicum* Wall and *Pharbitis nil* Chois.

The compounds, SK&F 525 and SK&F 3301, which inhibit the conversion of mevalonic acid to squalene, cause yellowing of treated leaves. Only the latter compound inhibits flowering.

Triparanol which inhibits the final steps of cholesterol synthesis, does not affect floral induction, but causes severe leaf necrosis. The symptoms are strictly localized, as is also true for the other inhibitors of this class.

The compounds, SK&F 7732 and SK&F 7997, which inhibit the conversion of lanosterol to cholesterol, are powerful inhibitors of floral induction of both Xanthium and Pharbitis.

SK&F 7997 is most active as a suppressor of flowering if applied to leaves shortly before the inductive long night. Application to buds or to leaves after a long night is without effect. The experimental results show that flower hormone synthesis is the process inhibited by SK&F 7997, and suggest that the hormone may be an isoprenoid- or steroid-like compound.

No substance has as yet been found capable of overcoming the inhibition caused by SK&F 7997.

Applications of labeled acetate or of mevalonic acid to leaves results in extensive incorporation of radioactivity into nonsaponifiable material both of leaves and buds. No differences could, however, be detected between patterns of incorporation by induced and noninduced plants.

In the presence of SK&F 7997, incorporation of radioactivity into the nonsaponifiable fraction by leaves is modified; two fractions, apparently of precursors, were found to accumulate and others to be diminished in amount. The precursors are converted by Xanthium leaves to a number of labeled compounds. A fraction whose formation is inhibited by SK&F 7997 contains β-sitosterol and stigmasterol. It may be concluded that SK&F 7997 inhibits sterol biosynthesis in Xanthium leaves.

β-Sitosterol and stigmasterol are synthesized in both vegetative and induced Xanthium leaves. The mechanism by which SK&F 7997 suppresses flowering cannot be by blocking biosynthesis of these two sterols.

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Literature Cited

1. Bennett, R. D. & E. Heftmann. 1962. Thin-layer chromatography of sterols. J. Chromatog: In press.
2. Bennett, R. D., E. Heftmann, A. E. Purcell, & J. Bonner. 1961. Biosynthesis of stigmasterol in tomato fruits. Science 134: 671–672.
3. Bergmann, W. 1953. The plant sterols. Ann. Rev. Plant Physiol. 4: 383–426.
4. Bonner, J. & J. A. D. Zeevaart. 1962. Ribonucleic acid synthesis in the bud an essential component of floral induction in Xanthium. Plant Physiol. 37: 43–49.
5. Chouard, P. 1936. Nouvelles recherches expérimentals sur l'action de la folliculine sur la floraison des Reines-Marguerites et de plusieurs autres plantes. Compt. rend. Soc. Biol. 122: 823–825.
6. Chouard, P. 1937. Action combinée de la folliculine et la durée d'éclairement sur la floraison des Reines-Marguerites. Compt. rend. Soc. Biol. 126: 509–512.
7. Hemberg, T. & B. Lowén. 1954. The effect of vitamin K & naphthalene acetic acid on Datura. Svensk farmaceutisk Tidskrift 58: 938–946.
8. Hendricks, S. B. 1960. The photoreactions controlling photoperiodism & related responses. In: Comparative Biochemistry of Photoreactive Systems, M. B. Allen, ed. Academic Press. Ch. 19: 303–321.
9. Heusner, A. 1958. Phytosterine. Encyl. Plant Physiol. X: 132–185.
10. Holmes, W. L. & N. W. DiTullio. 1962. Inhibitors of cholesterol biosynthesis which act at or beyond the mevalonic acid stage. Am. J. Clin. Nutr. 10: 310–322.
11. Marker, R. E., R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith, & C. H. Ruof. 1947. Steroidal sapogenins. J. Am. Chem. Soc. 69: 2167–2230.
12. Purcell, A. E., G. A. Thompson, Jr., & J. Bonner. 1959. The incorporation of mevalonic acid into tomato carotenoids. J. Biol. Chem. 234: 1081–1084.
13. Roberts, R. H. 1961. Photoperiodism, in view of recent lipid hormone induction studies. Plant Physiol. 36 suppl.: liii–liv.
14. Salisbury, F. B. 1955. The dual role of auxin in flowering. Plant Physiol. 30: 327–334.
15. Salisbury, F. B. 1959. Growth regulators & flowering. II. The cobaltous ion. Plant Physiol. 34: 598–604.
16. Sironval, C. 1957. La photopériode et la sexualisation du fraisier des quatre-saisons à fruits-rouges (Métabolisme chlorophyllien et hormone florigène). Compt. rend. de recherches (l'Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Bruxelles). 18: 1–229.
17. Zeevaart, J.A.D. 1962. DNA multiplication as a requirement for expression of floral stimulus in *Pharbitis nil*. Plant Physiol. 37: 296–304.
18. Zeevaart, J.A.D. 1962. Physiology of flowering. Science 137: 723–731.